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A MULTICENTER PROSPECTIVE RANDOMIZED PHASE II STUDY IN PATIENTS WITH CORTICOSTEROID-REFRACTORY/DEPENDENT/INTOLERANT CHRONIC CUTANEOUS GVHD GIVEN EITHER EXTRACORPOREAL PHOTOIMMUNE THERAPY WITH UVADEX® IN CONJUNCTION WITH CONVENTIONAL THERAPY OR CONVENTIONAL THERAPY ALONE

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Chronic GVHD (cGVHD) is a major complication of allogeneic hematopoietic stem cell transplantation (HSCT) and a leading cause of non-relapse mortality. Extracorporeal photoimmune therapy (ECP) has been used in retrospective and Phase II trials of patients with cGVHD. The mechanism of action of ECP is elusive but it appears to promote apoptosis and induce immune tolerance. In this randomized, prospective study, we compared the safety and efficacy of ECP used in conjunction with conventional therapy (calcineurin inhibitor ± mycophenolate mofetil) and steroids to conventional therapy alone in patients with steroid-refractory/dependent/intolerant cutaneous cGVHD. In the ECP arm, photopheresis was administered twice weekly for 12 weeks, then twice monthly until Week 24; patients in the non-ECP arm could withdraw from study after Week 12 if no beneficial response was observed or earlier if cGvHD worsened and enter a separate extension study that included ECP. The primary efficacy endpoint was percent change from baseline in skin assessment score (Total Skin Score; TSS) of 10 body regions graded from 0 to 5 (0 = normal, 1= discolored, 2 = lichenoid, 3 = thickened, 4 = hidebound, 5 = grades 3 or 4 with erythema; maximum score of 50) by a trained blinded observer at week 12. All efficacy analyses were conducted on the modified intent-to-treat (MITT) patients who had at least one treatment and one post-baseline TSS. Ninety-five MITT patients (56 M, 39 F; median age = 41 years) with steroid refractory (n = 12), intolerant (n = 30) or dependent (n = 57) histologically-confirmed cGVHD were randomized to receive ECP and conventional therapy (n = 48) or conventional therapy alone (n = 47). Study arms were balanced except for more GI involvement in the non-ECP arm. Ninety-four percent in both study arms had extensive cGVHD. 44 (92%) patients in the ECP arm and 41 (87%) in the non-ECP arm completed the first 12 weeks and those results are summarized in the table. ECP was well-tolerated with a safety profile consistent with previous studies. Thus, ECP is effective in skin manifestations and steroid sparing in steroid-refractory/dependent/intolerant chronic GVHD.

	ECP	Non-ECP	p Value
Median Baseline TSS	9.4	9.2	
Median % Improvement in TSS	14.5	10.4	0.510
Median % Improvement in pts with TSS >-10 at baseline	21.9	12.1	0.418
% pts with >-50% Reduction in Steroid Dose	29	14	0.089
% pts with >-50% Reduction in Steroid Dose AND final Steroid Dose < 10 mg/day	24	7	0.027
% pts with >-50% Reduction in Steroid Dose AND >-25% Reduction in TSS	10	0	0.040
% pts with CR and PR of Skin by Investigator Assessment	40	10	0.0024

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RAPID EXPANSION OF ACUTE MYELOID LEUKEMIA-REACTIVE CYTOTOXIC T CELLS FROM CD8+CD62L+ BLOOD LYMPHOCYTES OF HLA-MATCHED HEALTHY DONORS IN VITRO

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Allogeneic cytotoxic T-lymphocyte (CTL) therapy in acute myeloid leukemia (AML) is hampered by the poor efficiency in growing leukemia-reactive CTLs from healthy donors in vitro. We established an allogeneic mini-mixed lymphocyte-leukemia culture (MLLC) approach by stimulating comparably small numbers (10^4/well) of $CD8^+$ T cells isolated from peripheral blood of healthy donors against primary AML blasts in 96-well plates. Prior to use, $CD8^+$ T cells were immunomagnetically separated into a CD62L(high)$^+$ subset enriched for naive precursors and central memory cells as well as a CD62L(low)$^+$/negative subset containing effector memory cells. Mini-MLLCs were performed in seven healthy donor-AML pairs that were matched for HLA class I according to high-resolution sequence-based typing. Following 2 weekly re-stimulations with primary AML blasts, mini-MLLC responder populations were tested using split-well IFN-gamma ELISPOT assay. AML-reactive $CD8^+$ T-cell responders were obtained from all 7 donor-AML pairs with the majority of reactive cultures originally seeded with CD62L(high)$^+$ cells. In 4 out of 7 pairs most MLLC responder populations recognized AML blasts, but not Epstein-Barr virus transformed B-lymphoblastoid cell lines of donor and patient origin. This leukemia reactivity was restricted by various HLA class I alleles. Representative mini-MLLC responders demonstrated strong cytotoxicity against AML blasts in ^{51}Chromium-release assay. Cross-reactivity testing identified an HLA-A*0201-restricted CTL population that showed much stronger recognition of AML blasts compared to their non-malignant monocytic counterparts. This CTL did not recognize recipient-derived primary fibroblasts or other hematopoietic cells suggesting a leukemia-associated rather than a minor histocompatibility antigen as the target structure. Several MLLC-derived CTL populations expressed unique T cell receptor Vbeta chains consistent with clonal derivation from AML-reactive precursors. Multiple CTL responders reached a cell yield exceeding 10^8 by 6 to 10 weekly re-stimulations with AML blasts. Our results suggest that in healthy individuals most AML-reactive $CD8^+$ CTLs originate from the CD62L(high)$^+$ peripheral blood subpopulation containing naive precursor and central memory T cells. This mini-MLLC approach should allow the expansion of AML-reactive $CD8^+$ CTLs from HLA-matched healthy donors to cell numbers sufficient for antigen identification strategies or adoptive immunotherapy trials.

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AN INTEGRATED GENETIC-PROTEIN ANALYSIS OF THE TNF-FAMILY PATHWAY PREDICTS FOR GVHD

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Although the major cause of morbidity and treatment-related mortality (TRM) after allogeneic HSCT is GVHD, there is no predictive laboratory test for GVHD. Because tumor necrosis factor-α (TNF) plays an important role in GVHD pathogenesis we tested the hypothesis that elevations in TNF levels on day 7 would predict the development of significant GVHD and treatment-related mortality (TRM). We measured soluble TNF receptor 1 (TNFR1) as a surrogate for TNF because TNF circulates as a ligand-receptor complex. We studied samples obtained under informed consent from 438 patients undergoing allogeneic HSCT following myeloablative conditioning at the University of Michigan between 2000 and 2005. The median age of the patients was 42y (range 0-65y). The distribution of donors by degree of HLA-